

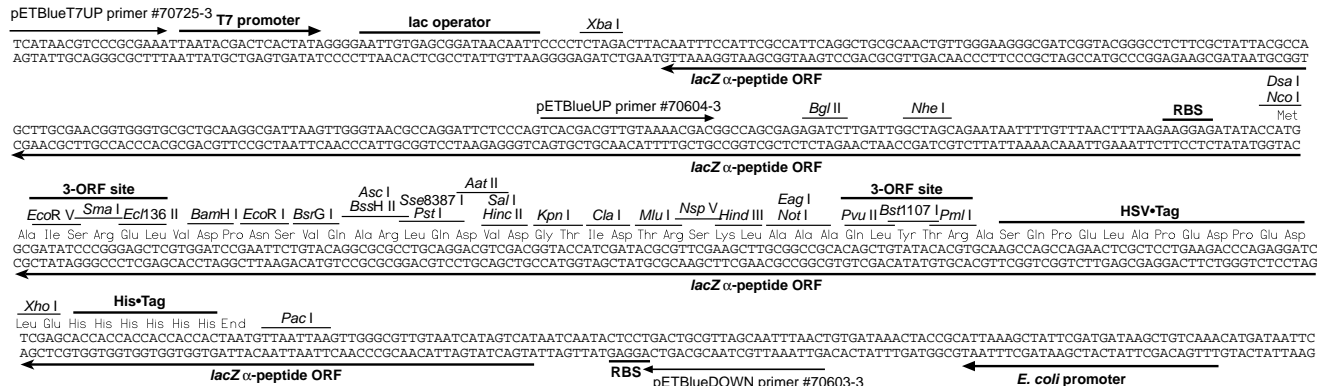
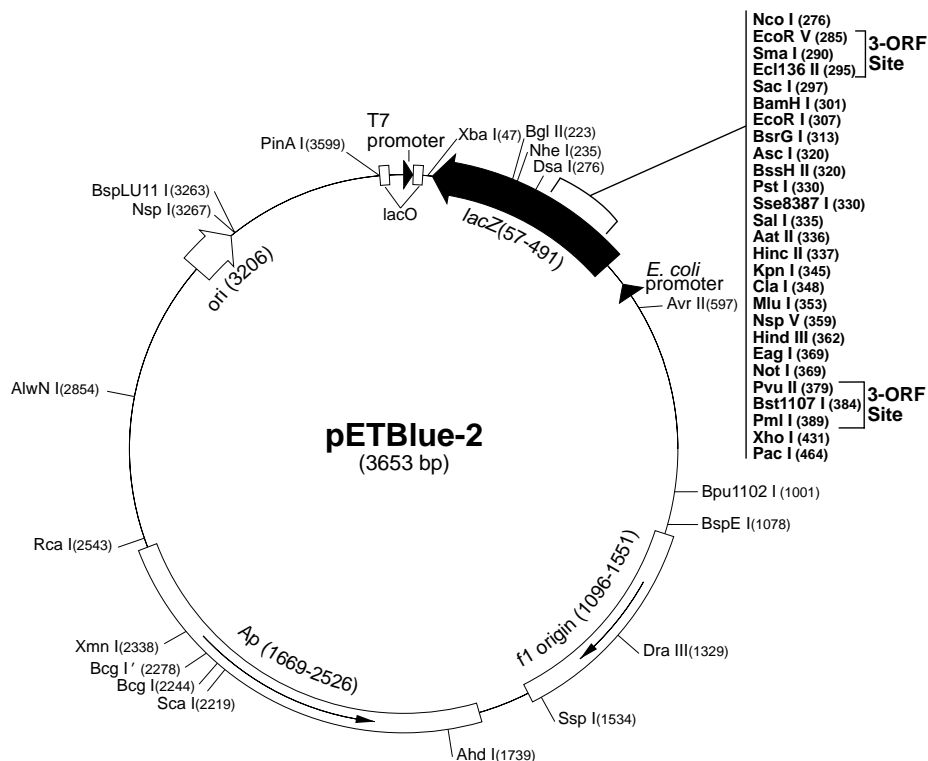
pETBlue-2 Vector

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The pETBlue™ vectors are designed to identify recombinants by traditional blue/white screening while also allowing T7lac promoter based expression of target genes. Screening is independent of expression because the T7lac expression promoter is in an opposed orientation relative to the *E. coli* promoter that mediates blue/white screening. pETBlue-2 defines the open reading frame and inserts must be cloned in-frame if expression is desired. The vector features an expanded multiple cloning site (MCS) and optional C-terminal HSV•Tag® and His•Tag® sequences. The cloning/expression region of the coding strand transcribed by T7 RNA polymerase is shown below. The sequence is numbered from the first base of the T7 promoter sequence. Unique sites are shown on the circle map. The f1 origin in pETBlue-2 is oriented so that infection with helper phage will produce virions containing single stranded DNA that corresponds to the coding strand. Therefore, single stranded sequencing should be performed using the pETBlueDOWN primer (Cat. No. 70603-3).

pETBlue-2 sequence landmarks

<i>lac</i> operator	3606–3625
T7 promoter	1–17
<i>lac</i> operator	22–42
T7 transcription start	18
multiple cloning region (<i>Nco</i> I– <i>Pac</i> I)	276–467
His•Tag® coding sequence	437–454
HSV•Tag® coding sequence	395–430
<i>lacZ</i> start codon	491
<i>lacZ</i> α-peptide ORF	57–491
<i>E. coli</i> promoter	541–569
f1 origin	1096–1551
<i>bla</i> coding sequence	1669–2526
pUC origin	3206



pETBlue-2 cloning/expression region

pETBlue-2 Restriction Sites

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Enzyme # Sites Locations	Enzyme # Sites Locations	Enzyme # Sites Locations
AatII 1 336	EcoRII 7 177 620 730 898 3102	Tsp45I 6 191 1148 1995 2206 3430
AccI 2 336 383	EcoRV 1 3115 3236	Tsp509I 18 3628
AcII 41	FauI 6 866 893 1118 1187 3415	TspRI 9 1687 1792 2139 2166 2584
AflIII 3 353 386 3263	Fnu4HI 25 3652	2855 2868 3374 3435
AhdI 1 1739	FspI 2 82 1961	VspI 1 1911
AluI 16	HaeII 6 688 1171 1179 3023 3540	XbaI 1 47
AlwI 11	HaeIII 20 3617	XhoI 1 431
Alw26I 2 709 1800	HgaI 5 890 1104 2265 2573 3151	XmnI 1 2338
AlwNI 1 2854	HhaI 24	
ApaLI 2 2406 2949	HincII 1 337	
ApoI 4 307 614 1509 1520	HindIII 1 362	
AscI 1 320	HinfI 9 7 182 1374 1396 1734	
AvaI 2 288 431	2893 3289 3364 3460	
Avall 5 641 1877 2099 3425 3513	HphI 8 810 1326 1582 1809 2225	
AvrII 1 597	2431 2466 3614	
BamHI 1 301	KpnI 1 345	
BanI 4 341 1285 1687 3545	MaeIII 14	
BanII 2 297 1255	MbolI 10 107 428 596 1187 1579	
BbsI 3 423 591 3581	2334 2412 2521 2606 3586	
BbvI 12	MluI 1 353	
BcgI 1 2244	MnII 18	
BcgI' 1 2278	MseI 23	
Bfal 8 48 236 598 1012 1173	MslI 3 1991 2150 2509	
1594 1929 2770	MspI 17	
BglI 2 75 1859	MspA1I 5 379 1000 2374 2680 2925	
BglII 1 223	MwoI 15	
Bpml 2 1809 3485	NciI 7 289 290 830 863 1922	
Bpu1102I 1 1001	2273 2886	
BsaI 2 709 1800	NcoI 1 276	
BsaAI 2 389 1326	NgoAIV 3 1221 3382 3542	
BsaHI 3 333 882 2276	NheI 1 235	
BsaJI 5 276 288 597 1023 3103	NlaIII 12	
BsaWI 5 1078 2030 2910 3057 3599	NlaIV 15	
BsiEI 7 103 372 2109 2258 2929	NotI 1 369	
3353 3600	NspI 1 3267	
BsiHKAI 5 297 438 2325 2410 2953	NspV 1 359	
BsII 8 869 1037 1107 1433 2785	PacI 1 464	
3064 3230 3248	PinAI 1 3599	
BsmFI 4 627 1107 3438 3627	PleI 6 1 1382 1390 1728 2887	
Bsp1286I 6 297 438 1255 2325	3372	
2410 2953	PmlI 1 389	
BspEI 1 1078	Psp1406I 5 845 1539 1965 2338	
BspLU11I 1 3263	3412	
BsrI 12	PstI 1 330	
BsrBI 4 30 835 1182 3334	PvuI 2 103 2109	
BsrDI 2 1800 1974	PvuII 1 379	
BsrFI 5 1221 1819 3382 3542 3599	RcaI 1 2543	
BsrGI 1 313	RsaI 4 107 315 343 2219	
BssHII 1 320	SacI 1 297	
BssSI 4 296 2403 3090 3626	Sall 1 335	
Bst1107I 1 384	Sau3AI 18	
BstYI 9 223 301 427 1584 1596	Sau96I 13	
2364 2381 2611 2622	Scal 1 2219	
CacBI 24	ScrFI 14	
Clal 1 348	SfaNI 6 744 912 1995 2186 2435	
CviJI 58	3166	
Ddel 8 665 755 811 1001 1696	Sfcl 6 13 326 1103 1980 2807	
2236 2580 2989	2998	
DpnI 18	SmaI 1 290	
DraI 3 1605 1624 2316	Sse8387I 1 330	
DraIII 1 1329	Sspl 1 1534	
DrdI 2 1373 3161	Styl 3 276 597 1023	
Dsal 1 276	Tail 14	
Ecl136II 1 295	TaqI 9 336 348 359 432 552	
EaeI 3 212 369 2127	760 1291 2390 3165	
EagI 1 369	TfiI 3 182 3289 3460	
EarI 2 120 2534	ThaI 13	
Eco47III 2 3538 3615	TseI 12	
Eco57I 3 435 2406 2721		
EcoO109I 2 594 1028		
EcoRI 1 307		

Enzyme that do not cut pETBlue-2:

AflII	Apal	BclI	Bpu10I	BsaBI	BseRI
BsgI	BsmI	BsmBI	BspMI	BstEII	BstXI
Bsu36I	EcoNI	FseI	HpaI	MscI	MunI
NarI	NdeI	NruI	Nsil	PfiI	PmeI
PshAI	Psp5II	RsrII	SacII	SanDI	SapI
SexAI	SfiI	SgfI	SgrAI	SnaBI	SpeI
SphI	SrfI	StuI	SunI	Swal	Tth111I
UbaEI	XcmI				